ISSN 1346-7565

Acta Phytotax. Geobot. 68 (1): 1–15 (2017)
doi: 10.18942/apg.201617

Genetic Structure of Remnant *Quercus serrata* Populations at the Northernmost Limit of their Distribution in Japan

Keiko Kitamura[1,*], Kanji Namikawa[2], Takayuki Kawahara[3], Asako Matsumoto[3] and Lerma San Jose-Maldia[4]

*[1] Hokkaido Research Center, Forestry and Forest Products Research Institute, 7 Hitsujigaoka, Toyohira-ku, Sapporo 062-8516, Japan. *kitamq@ffpri.affrc.go.jp (author of correspondence); [2]Biological Laboratory, Sapporo Campus, Hokkaido University of Education, Sapporo 002-8502 Japan; [3]Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan; [4]University of the Philippines Los Baños, College 4031, Laguna, Philippines*

The northernmost limit of distribution of *Quercus serrata* is on Hokkaido, where fragmented populations occur from the southwestern foothills of the Hidaka Mountains to the Ishikari Plain with an additional isolated population on the Oshima Peninsula. These northern marginal populations are considered to be the result of past vegetational shifts, but the genetic relationships are not fully understood. In this study, we used 11 nuclear SSR loci to genotype 667 individuals from 11 populations of *Q. serrata* in Hokkaido and six populations from the Tohoku region of Honshu. Total genetic diversity in Hokkaido (H_E = 0.693) did not differ from Tohoku (H_E = 0.669). The population differentiation in Hokkaido (G'_{ST} = 0.097) was not significantly different from that of Tohoku (G'_{ST} = 0.090). The STRUCTURE analysis distinguished four regional clusters of *Q. serrata*; the Pacific side of Tohoku, the Japan Sea side of Tohoku, the northernmost region, and the Oshima Peninsula. The findings indicate two ancestral origins from the Pacific and Japan Sea coasts of Tohoku in the Hidaka region. The results from principal coordinate analyses indicated that the isolated population on the Oshima Peninsula is more related to the populations on Hokkaido than to the Tohoku populations.

Key words: Fagaceae, fragmented distribution, northern distribution range, nuclear microsatellite, STRUCTURE analysis

The distribution of plant species is determined by several factors, such as physiological limitations, ecological niche, and geological history (Heuertz *et al*. 2004). Plant populations in the center of their distribution generally sustain stable conditions in a favorable environment for the species, while sites at the margin of their distribution are exposed to less suitable sites for establishment and to greater competition with other species than at their distribution center (Lawton 1993, Hampe & Petit 2005). Because of these disadvantages, local populations at the margins of their range of distribution often occur in small, isolated populations (Hampe & Petit 2005, Kitamura *et al*. 2015). The present distribution of plant species also provides clues to their past migration history, which is influenced by various factors such as changes in the environment, geological activity, competition with other species, and interaction between biotic and abiotic factors. During migration, distributional changes such as expansion, contraction, fusion and isolation inevitably result in changes in genetic variation by chance and/or by natural selection. Therefore, the effects of the past reflect on the genetic relationships among current populations (McLachlan *et al*. 2005, Magri *et al*. 2006).

Quercus serrata Murray (Fagaceae), is widely distributed throughout the warm temperate and lower cool temperate zones of the Japanese Archipelago from Kyushu to Hokkaido and the Korean Peninsula, often intermixed with other

deciduous broadleaved species of trees. It grows between 100 and1000 m elevation and is better adapted to a warmer and drier environment than its sister species, *Q. crispula* (syn. *Q. mongolica* var. *crispula*) in the northern part of its range in northern Tohoku and southern Hokkaido. *Quercus serrata* is less frequent in central Hokkaido, where it is at the northernmost extent of its natural distribution.

Uemura & Takeda (1987) investigated the distribution, ecological characteristics and climatic conditions of 129 cool temperate forest species in Hokkaido and classified their distribution patterns into 8 chorological groups represented by the major plant species; (A) *Fagus crenata* group, (B) *Aesculus turbinata* group, (C) *Arachniodes standishii* group, (D) *Leptorumohra miqueliana* group, (E) *Cacalia delphiniifolia* group, (F) *Carpinus laxiflora* group (G) *Ostrya japonica* group, and (H) whole area group. In this classification, *Q. serrata* belongs to the Pacific Ocean side distribution of (E), the *Cacalia delphiniifolia* group, with *Neillia incisa* (Thunb.) S. H. Oh, *Acer cissifolium* (Siebold & Zucc.) K. Koch, *Callicarpa japonica* Thunb., *Spuriopimpinella calycina* (Maxim.) Kitag., and *Zanthoxylum piperitum* (L.) DC. This group has a discontinuous distribution on the Oshima Peninsula, Hidaka, and Iburi regions. Uemura & Takeda (1987) attributed the distribution of this group to an environment where there is > 50 cm of snow depth for 30 days per year.

Recent progress in DNA markers combined with paleobotanical evidence has contributed to the reconstruction of migration routes after the last glacial maximum in the northern hemisphere (Hewitt 2000, Comes & Kadereit 1998, Taberlet *et al*. 1998, Stewart & Lister 2001, Petit *et al*. 2002, Lascoux *et al*. 2004, Lumaret *et al* 2005, Magni *et al*. 2005, McLachlan *et al*. 2005, Magri *et al*. 2006). The phylogeography of Japanese oaks has been investigated for both the entire and parts of their geographic range (Ubukata 2003, Kanno *et al*. 2004, Okaura *et al*. 2007, Quang *et al*. 2008, Harada & Ikeda 2011, Ohsawa *et al*. 2011). Most of these studies have discussed the major species, *Q. crispula*, while few have focused on *Q. serrata*. The studies of Kanno (2004) revealed that *Q. serrata* in Hokkaido was monophyletic and showed low genetic diversity in nuclear simple sequence repeats (nSSRs), but the genetic relationships among populations of *Q. serrata* in its northernmost range of distribution have not been investigated in detail.

In general, sympatric species of oaks may form natural hybrids that result in introgression between pairs of species (Whittemore & Schaal 1991, Lee *et al*. 1996, Ishida *et al*. 2003). We noted that the closely related *Q. crispula* grows within or adjacent to *Q. serrata* in the northern part of its range of distribution. A study by Okaura *et al*. (2007) reported that four sympatric species of oaks in the Japanese Archipelago, including two cpDNA haplotypes shared among sympatric *Q. serrata* and *Q. crispula* in Hokkaido, shared identical cpDNA haplotypes, indicating the possibility of occasional introgression among the species.

In this study, we focused on the genetic structure of *Q. serrata* in Hokkaido northernmost limit of distribution and compared it with populations from Tohoku. We discuss the consequences of introgression from sympatric *Q. crispula* to *Q. serrata* and elucidate (i) the genetic diversity and differentiation at the northernmost distribution limit of *Q. serrata* and (ii) the genetic relationships of the isolated population on the Oshima Peninsula.

Materials and Methods

Study sites

Eleven natural populations of *Q. serrata* across the range of distribution in Hokkaido were analyzed; two populations from the northernmost limit of its range (1-Takikawa, 2-Mikasa), two from the Ishikari Plain (3-Oyafuru, 4-Ohyachi), six from the Hidaka region (5-Atsuma, 6-Hobetsu, 7-Hirotomi, 8-Shin-ei, 9-Utafue, and 10-Kineusu), and one from the Oshima Peninsula (11-Ohnuma) (Fig. 1, Table 1). Voucher specimens were deposited in the herbarium of the Graduate School of Science, Hokkaido University (SAP) (Table 1). In two isolated populations at the northern limit of its range, *Q. serrata* is dominant, but mixed with *Q. crispula*, *Acer japoni-*

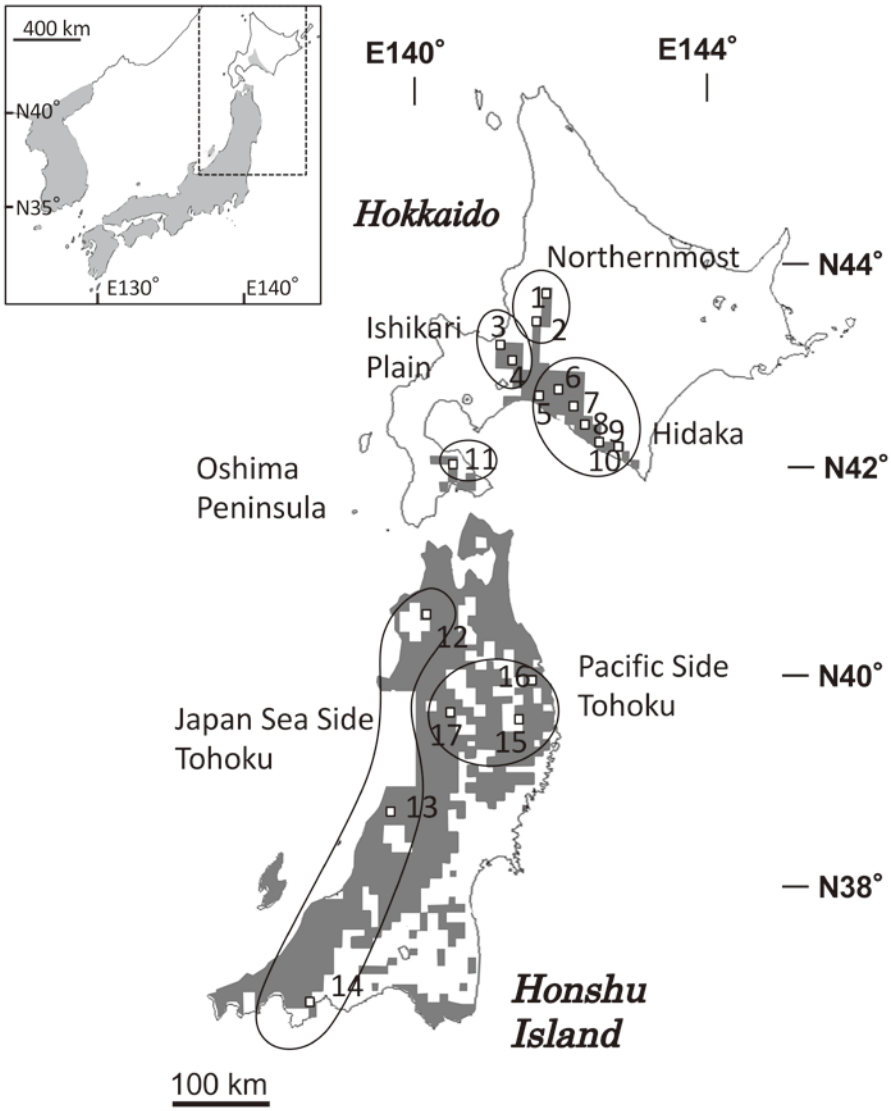

FIG.1. Geographic distribution of *Quercus serrata* in Japan and Korea showing the 17 populations analyzed in this study (lower right). Numbers correspond to Table 1; Northernmost, Ishikari Plain, Hidaka, Oshima Peninsula, and Tohoku.

cum, *Tilia maximowicziana* var. *yezoana*, and *Sasa senanensis*. Two study sites on the Ishikari Plain are also isolated. They are lowland populations mixed with *Q. crispula*, *Acer japonicum*, *Magnolia obovata*, *M. kobus* var. *borealis*, *Aria alnifolia*, and *Ostrya japonica*. The Hidaka region has the major concentration of *Q. serrata* on Hokkaido (Tohyama & Mochida 1978). The continuous populations in the Hidaka region are mixed forests of *Q. crispula*, *Carpinus laxiflora*, *Acer pictum* (syn. *A. mono*), *Sasa nipponica*, and *Cacalia delphiniifolia*. One, remote, isolated population of *Q. serrata* is on the Oshima Peninsula, where it is mixed with *Q. crispula*, *Acer amoenum* var. *matsumurae*, and *Aria alnifolia*; the ground cover is *Sasa senanensis*.

Six populations from the Tohoku region, northern Honshu, were selected as references; the Japan Sea side of Tohoku (12-Hirosaki, 13-Tsuruoka, 14-Tokamachi) and the Pacific side of Tohoku (15-Iwaizumi, 16-Hayachine, 17-Tazawako) (Fig. 1, Table 1).

TABLE 1. Location and genetic diversity of the 11 natural *Q. serrata* Hokkaido populations and six references from northern Honshu analyzed in this study.

Population	Region	Latitude (N)	Longitude (E)	Altitude (m)	Voucher	*n*	*Ne*	*PA*	*AR*	H_O	H_E	F_{IS}[a]	LD[b]	*P* value[c]		
														TPM	IAM	SMM
Populations in Hokkaido																
1 - Takikawa	Northernmost	43°32'13"	141°56'23"	101.7	*SAPS 049785, 049790*	49	3.757	2	5.959	0.683	0.683	0.000	0.255	0.711	0.074	0.973
2 - Mikasa	Northernmost	43°14'51"	141°50'25"	118.3	*SAPS 049781, 049783*	50	3.677	2	5.986	0.658	0.693	0.050 *	0.327	0.260	0.002	0.997
3 - Oyafuru	Ishikari Plain	43°11'35"	141°22'14"	6.0	*SAPS 049786, 049787*	48	3.990	3	6.247	0.699	0.716	0.024	0.436	0.319	0.001	0.995
4 - Ohyachi	Ishikari Plain	43°01'23"	141°27'00"	33.0	*SAPS 049791, 049792*	48	3.577	0	5.978	0.696	0.691	-0.006	0.436	0.318	0.002	0.997
5 - Atsuma	Hidaka	42°38'10"	141°53'28"	11.9	*SAPS 049773, 049774*	49	3.503	1	6.065	0.696	0.686	-0.015	0.291	0.897	0.027	1.000
6 - Hobetsu	Hidaka	42°45'47"	142°07'58"	94.7	*SAPS 049788, 049789*	25	3.442	1	5.611	0.665	0.652	-0.020	0.364	0.551	0.120	0.938
7 - Hirotomi	Hidaka	42°35'29"	142°14'37"	96.0	*SAPS 049772, 049784*	85	3.944	2	6.274	0.675	0.699	0.034 *	0.327	0.840	0.067	0.999
8 - Shin-ei	Hidaka	42°27'39"	142°24'36"	48.5	*SAPS 049778, 049779*	20	3.935	0	6.286	0.736	0.693	-0.063	0.182	0.517	0.027	0.897
9 - Utafue	Hidaka	42°16'44"	142°39'50"	38.6	*SAPS 049771, 049775*	49	3.996	5	6.433	0.694	0.707	0.019	0.364	0.416	0.001	0.997
10 - Kineusu	Hidaka	42°14'40"	142°54'29"	74.7	*SAPS 049776, 049777*	50	3.934	0	6.056	0.707	0.709	0.002	0.145	0.120	0.000	0.973
11 - Ohnuma	Oshima Peninsula	42°00'16"	140°41'08"	147.4	*SAPS 049780, 049782*	50	3.647	2	5.436	0.729	0.699	-0.044 *	0.327	0.103	0.000	0.956
Total Hokkaido							3.764			0.694	0.693	-0.018				
Populations in Honshu																
12 - Hirosaki	Japan Sea side of Tohoku	40°33'36"	140°19'14"	217.4	--	28	3.562	2	6.133	0.623	0.658	0.052	0.073	0.897	0.289	0.998
13 - Tsuruoka	Japan Sea side of Tohoku	38°45'45"	139°44'52"	271.2	--	28	4.210	3	6.742	0.692	0.721	0.040	0.255	0.207	0.034	0.997
14 - Tokamachi	Japan Sea side of Tohoku	37°05'59"	138°37'02"	314.5	--	16	3.567	0	6.182	0.699	0.691	-0.012	0.073	0.740	0.120	0.973
15 - Iwaizumi	Pacific side of Tohoku	40°01'19"	141°47'24"	172.1	--	24	3.240	0	5.854	0.629	0.628	-0.037	0.145	0.768	0.289	0.990
16 - Hayachine	Pacific side of Tohoku	39°36'49"	141°30'54"	568.6	--	24	3.535	4	6.291	0.617	0.660	-0.034	0.400	0.618	0.350	0.997
17 - Tazawako	Pacific side of Tohoku	39°42'30"	140°41'24"	289.5	--	24	3.256	1	5.929	0.640	0.660	0.007	0.145	0.990	0.207	1.000
Total Honshu							3.562			0.667	0.669	-0.019				

n: sample size; *Ne:* number of effective alleles per locus; *PA:* number of private alleles; *AR:* allelic richness based on 16 individuals; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: fixation index

[a] Deviations of F_{IS} from 0 in each population were tested by permutation tests (*$P < 0.05$, **$P < 0.01$)

[b] LD, the percentage of significant ($P < 0.05$) linkage disequilibrium out of 55 pairs of loci

[c] Probabilities associated with the Wilcoxon's signed rank tests (one-tailed) for H_E excess from H_{EQ} under two-phase model (TPM), infinite allele model (IAM), and stepwise mutation model (SMM)

TABLE 2. Genetic diversity for 11 SSR loci in *Q. serrata*.

Locus name	TA	H_T	H_S	H_O	F_{IS}	F_{ST}	R_{ST}
bcqm07	23	0.866	0.840	0.853	-0.032	0.029	0.024
bcqm27	10	0.567	0.544	0.553	-0.040	0.036	0.024
bcqm31	10	0.706	0.681	0.698	-0.033	0.035	0.039
bcqm42	9	0.722	0.714	0.707	-0.008	0.012	0.034
bcqm50	6	0.626	0.610	0.601	-0.002	0.031	0.054
bcqm74	12	0.744	0.724	0.701	0.015	0.028	0.028
bcqm76	27	0.874	0.861	0.831	0.020	0.015	0.041
bcqm94	9	0.402	0.394	0.413	-0.061	0.019	0.031
bcqm325	14	0.751	0.728	0.740	-0.034	0.032	0.014
QpZAG36	10	0.779	0.758	0.775	-0.038	0.034	0.048
QpZAG46	16	0.748	0.681	0.661	0.011	0.096	0.145
Overall		0.708	0.685	0.685	-0.018	0.034	0.051

TA: Total number of alleles; H_T: gene diversity in the total population; H_S: average gene diversity within populations; H_O: observed heterozygosity. F_{IS} fixation index, F_{ST} and R_{ST} measures of relative gene differentiation among populations defined under the infinite allele model (IAM) and the stepwise mutation model (SMM), respectively.

Sample collection, DNA extraction, and SSR analysis

Inner bark tissue and leaves were collected from 16 to 50 randomly chosen mature trees (diameter at breast height, DBH > 20.0 cm) from each population (Table 1). Sample tissues were stored below 5°C until DNA extraction.

Thirty milligrams of sample tissue were frozen with liquid nitrogen and ground to a powder with a Multi-beads Shocker (Yasui Kikai Co., Ltd, Shiga, Japan). DNA was extracted using the DNeasy Plant Mini Kit (Qiagen Inc., Tokyo, Japan). We employed 11 SSR markers, QpZAG36, QpZAG46 (Steinkellner *et al*. 1997), bcqm07, bcqm27, bcqm31, bcqm42, bcqm50, bcqm74, bcqm76, bcqm94, and bcqm325 (Mishima *et al*. 2006) (Table 2). Polymerase chain reaction (PCR) was performed with a Multiplex PCR Kit (Qiagen), denatured at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 57°C for 90 sec, and extension at 72°C for 1 min, and a final extension incubation of 30 min at 60°C. Fragment analyses were performed with an ABIPRISM 3130xl Genetic Analyzer (Life Technologies Japan, Tokyo, Japan). Fragment sizes were determined by the GeneScan 500-LIZ size standard (Life Technologies Japan) and an ABI GeneMapper ver. 4.1 (Life Technologies Japan). Genotyping data have been deposited in the TreeGenes Database under accession number TGDR047.

Data analysis

The following genetic diversity parameters were calculated in FSTAT ver. 2.9.3.2 (Goudet 1995): the number of effective alleles per locus (*Ne*), allelic richness (*AR*) based on a minimum sample size of 16, observed (H_O), the total population (H_T), within populations heterozygosity (H_S), Nei's unbiased expected heterozygosity (H_E), and the inbreeding coefficient (F_{IS}). The significance of deviation of F_{IS} from zero was evaluated on the basis of 1,000 randomizations. Genetic differentiation among populations was estimated by F_{ST}, and R_{ST}. Pairwise F_{ST} was also calculated, and its significance was tested on the basis of 1,000 randomizations, after Bonferroni correction.

We used GenoDive ver. 2.0b23 (Meirmans & Van Tienderen 2004) to calculate G'_{ST} (Hedrick 2005) and compare differences in genetic diversity parameters (Goudet 1995) between Tohoku and Hokkaido regions; the test was performed under 999 permutations by randomizing the populations over the regions.

The program Arlequin ver. 3.5 (Excoffier *et al*. 2005) was used to test the linkage disequilib-

rium (LD) within each pair of loci and populations by 10,000 permutations.

GenAlEx ver. 6.5 (Peakall & Smouse 2006) was used to calculate the number of private alleles (*PA*) for the 17 populations and isolation by distance (IBD) by simple regression analysis with a matrix of pairwise population differentiation in terms of $F_{ST} / (1 - F_{ST})$. The significance was evaluated by the Mantel test based on 999 permutations (Mantel 1967). Principal coordinate analysis (PCoA) of 17 populations was conducted based on pairwise F_{ST} among populations using GenAlEx.

To evaluate recent bottlenecks, we employed a two-tailed Wilcoxon signed-rank test using Bottleneck ver. 1.2.02 (Piry *et al*. 1999) under the two-phase model (TPM), the infinite allele model (IAM) or the stepwise mutation model (SMM).

STRUCTURE ver. 2.3.4 (Pritchard *et al.* 2000) was used to understand the components of genetic ancestry among populations. Analyses were based on an admixture model and correlated allele frequencies model, with prior information on location (hereafter, the *F*-model). Simulations were carried out 10 times for each $K = 1$–10 value with 70,000 burn-ins and 30,000 MCMC iterations. The log likelihood, $\ln P(X|K)$, and ΔK were calculated in STRUCTURE Harvester (Earl & vonHoldt 2012) to estimate the most likely number of clusters (K). The STRUCTURE results were summarized in CLUMPP_Windows 1.1.2 software (Jakobsson & Rosenberg 2007). Mean *F* values (equivalent to F_{ST}) for each cluster and the genetic distance among clusters was calculated from the results of 10 runs, considering label switching among the runs.

Results

Genetic diversity of northern populations of Q. serrata

The genetic diversity of the 11 SSR loci is summarized in Table 2. Gene diversity of the total population (H_T) and the average gene diversity within populations (H_S) was 0.708 and 0.685, respectively. The observed heterozygosity (H_O) ranged from 0.413 at bcqm94 to 0.853 at bcqm07. The value of F_{IS} ranged from −0.061 (bcqm94) to 0.020 (bcqm76) (Table 2).

Table 1 shows the genetic variation in the 17 populations. The number of effective alleles (*Ne*) ranged from 3.442 (6-Hobetsu) to 3.996 (9-Utafue) in Hokkaido and from 3.240 (15-Iwaizumi) to 4.210 (13-Tsuruoka) in Tohoku. The number of private alleles (*PA*) for the population of 9-Utafue was 5, which was the highest in the populations we examined from Hokkaido. The allelic richness *(AR*) ranged from 5.436 (11-Ohnuma) to 6.286 (8-Shin-ei) in Hokkaido, and from 5.854 (15-Iwaizumi) to 6.742 (13-Tsuruoka) in Tohoku. H_O ranged from 0.658 (2-Mikasa) to 0.736 (8-Shin-ei) in Hokkaido and from 0.617 (16-Hayachine) to 0.699 (14-Tokamachi) in Tohoku; the expected heterozygosity (H_E) ranged from 0.652 (6-Hobetsu) to 0.716 (3-Oyafuru) in Hokkaido and from 0.628 (15-Iwaizumi) to 0.721 (13-Tsuruoka) in Tohoku. The F_{IS} values ranged from −0.063 (8-Shin-ei) to 0.050 (2-Mikasa). Three populations deviated significantly from zero (2-Mikasa, 6-Hirotomi, and 11-Ohnuma).

The proportion of significant ($P < 0.05$) linkage disequilibrium (LD) among 55 pairs of loci within populations ranged from 0.073 (12-Hirosaki and 14-Tokamachi) to 0.436 (3-Oyafuru and 4-Ohyachi) (Table 1). Mishima *et al*. (2006) reported significant LD for 4 pairs of 6 loci which we used in this study. However, the observed bias of LD was not severe, even if the SSRs were partially linked, as the following results of the STRUCTURE analysis was similar to the PCoA, which was free from genetic assumptions.

Genetic differentiation among populations

The genetic differentiation among populations, F_{ST}, R_{ST}, and G'_{ST}, was 0.034, 0.051, and 0.106, respectively (Table 2). The genetic differentiation G'_{ST} in Hokkaido (0.097) was comparable ($P = 0.739$) to that of the Tohoku region ($G'_{ST} = 0.090$). Pairwise F_{ST} values for all population pairs, except for 8-Shin-ei to 9-Utafue and 12-Hirosaki to 14-Tokamachi, showed significant differentiation (Table 3).

The Bottleneck analysis results did not show any significant excess of heterozygosity ($P <$

TABLE 3. Pairwise F_{ST} (below diagonal) and its significance (upper diagonal) among 17 *Q. serrata*

	1 Takikawa	2 Mikasa	3 Oyafuru	4 Ohyachi	5 Atsuma	6 Hobetsu	7 Hirotomi	8 Shin-ei	9 Utafue	10 Kine-usu	11 Ohnuma	12 Hirosaki	13 Tsuruoka	14 Tokamachi	15 Iwaizumi	16 Hayachine	17 Tazawako
1 Takikawa	--	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
2 Mikasa	0.016	--	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
3 Oyafuru	0.033	0.018	--	**	**	**	**	**	**	**	**	**	**	**	**	**	**
4 Ohyachi	0.032	0.021	0.018	--	**	**	**	**	**	**	**	**	**	**	**	**	**
5 Atsuma	0.031	0.020	0.017	0.021	--	**	**	**	**	**	**	**	**	**	**	**	**
6 Hobetsu	0.020	0.020	0.025	0.030	0.020	--	**	**	**	**	**	**	**	**	**	**	**
7 Hirotomi	0.039	0.037	0.026	0.048	0.029	0.032	--	**	**	**	**	**	**	**	**	**	**
8 Shin-ei	0.045	0.036	0.022	0.042	0.017	0.030	0.016	--	n. s.	**	**	**	*	**	**	**	**
9 Utafue	0.048	0.037	0.022	0.037	0.021	0.027	0.035	0.006	--	**	**	**	**	**	**	**	**
10 Kine-usu	0.033	0.025	0.017	0.024	0.012	0.023	0.027	0.022	0.023	--	**	**	**	**	**	**	**
11 Ohnuma	0.064	0.050	0.033	0.052	0.043	0.059	0.044	0.040	0.043	0.031	--	**	**	**	**	**	**
12 Hirosaki	0.061	0.051	0.047	0.060	0.046	0.048	0.012	0.024	0.045	0.054	0.054	--	**	n. s.	**	**	**
13 Tsuruoka	0.044	0.037	0.029	0.040	0.030	0.033	0.014	0.011	0.029	0.030	0.041	0.013	--	*	**	**	**
14 Tokamachi	0.053	0.048	0.041	0.048	0.043	0.039	0.018	0.025	0.043	0.047	0.045	-0.001	0.017	--	**	**	**
15 Iwaizumi	0.042	0.048	0.037	0.040	0.038	0.026	0.050	0.037	0.041	0.056	0.058	0.052	0.038	0.043	--	**	**
16 Hayachine	0.033	0.036	0.025	0.038	0.039	0.025	0.044	0.031	0.032	0.034	0.049	0.048	0.028	0.047	0.028	--	**
17 Tazawako	0.037	0.028	0.025	0.038	0.018	0.025	0.037	0.022	0.021	0.028	0.046	0.047	0.020	0.046	0.028	0.018	--

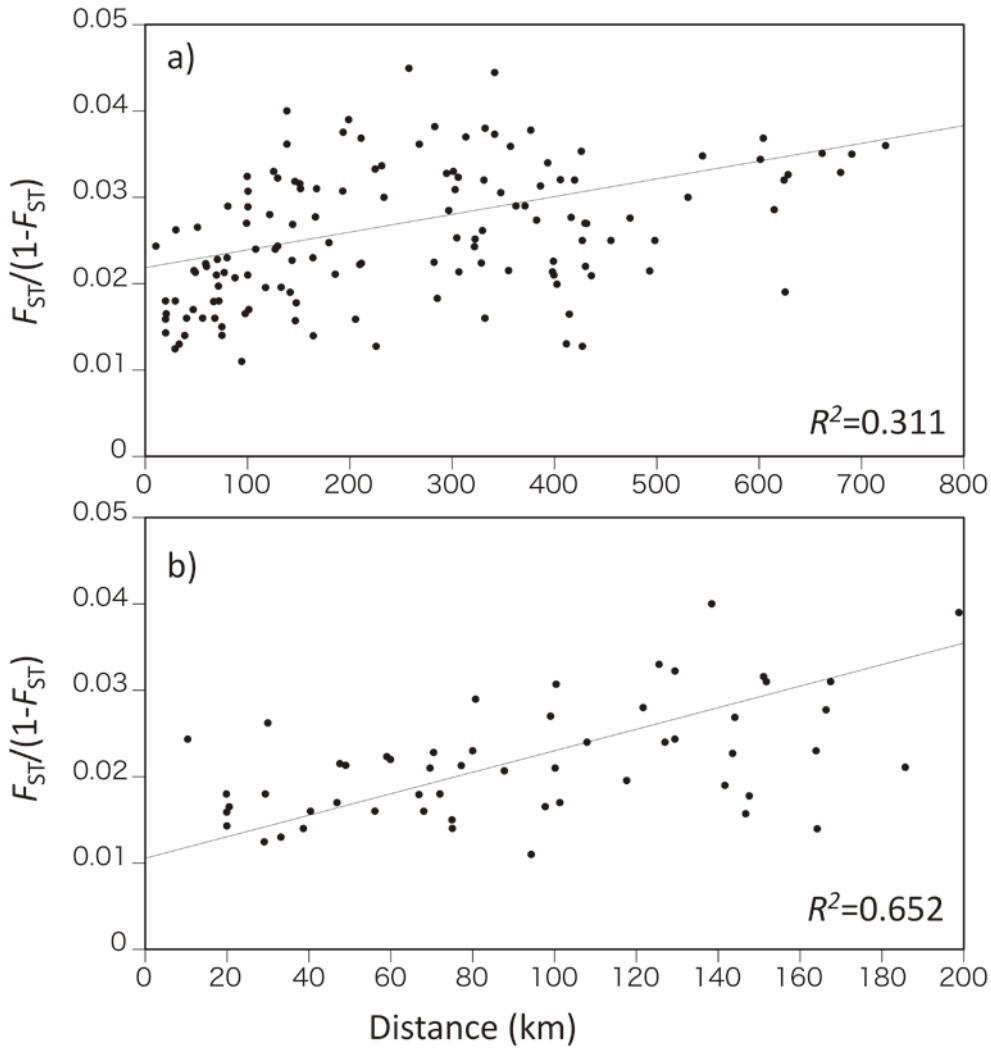

FIG.2. Relationship between pairwise differentiation, $F_{ST} / (1 - F_{ST})$, and geographic distance (km) among populations of *Q. serrata*; a) all 17 populations, b) 11 populations in Hokkaido.

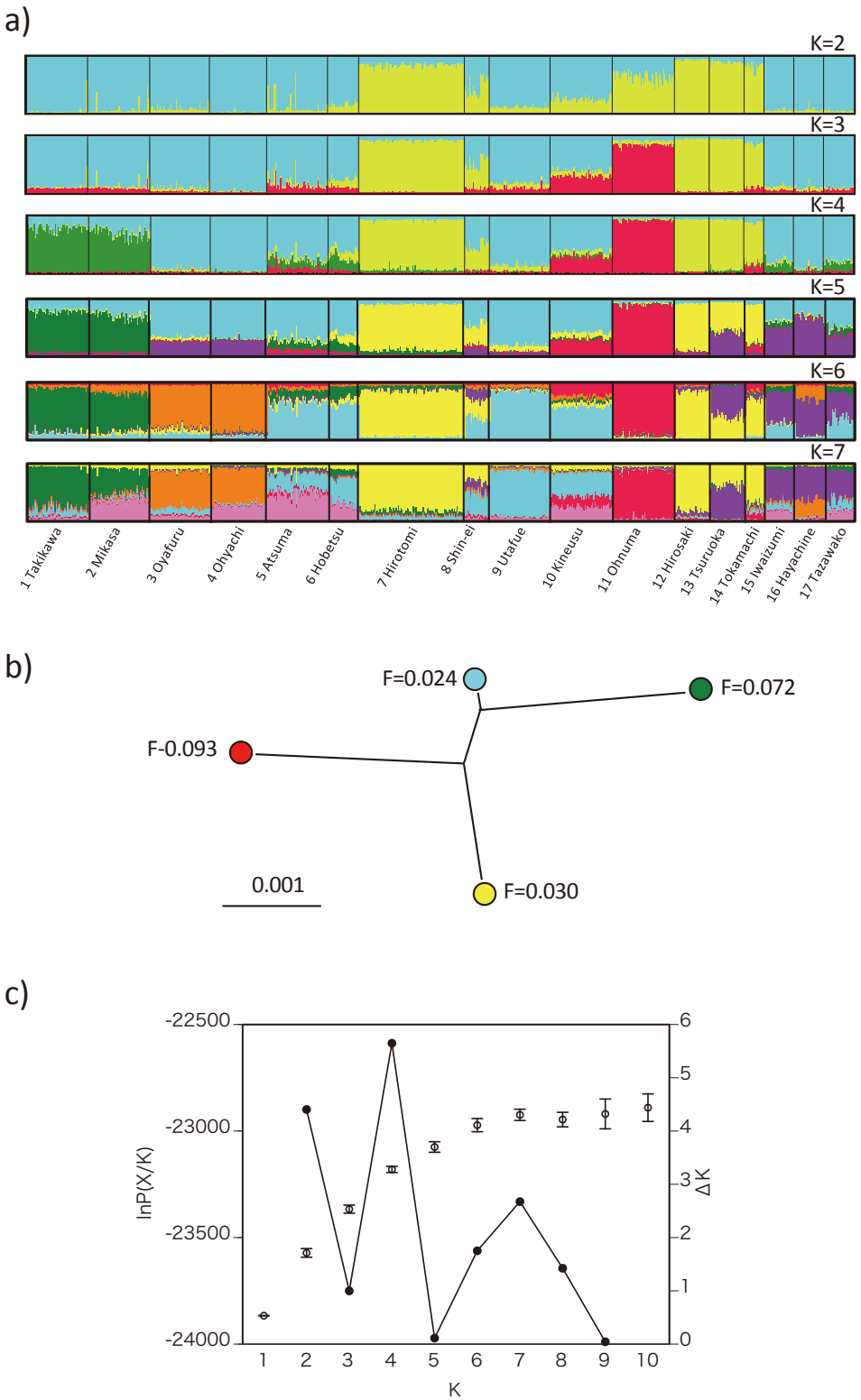

FIG.3. Structure results for 17 populations of *Q. serrata*. (a) Bar plots for $K = 2$ to 7. (b) Neighbor-joining tree for each cluster when $K = 4$ along with F values for each cluster. (c) Log likelihood for each K value. Open circles, ln $P(X/K)$; filled circles, ΔK.

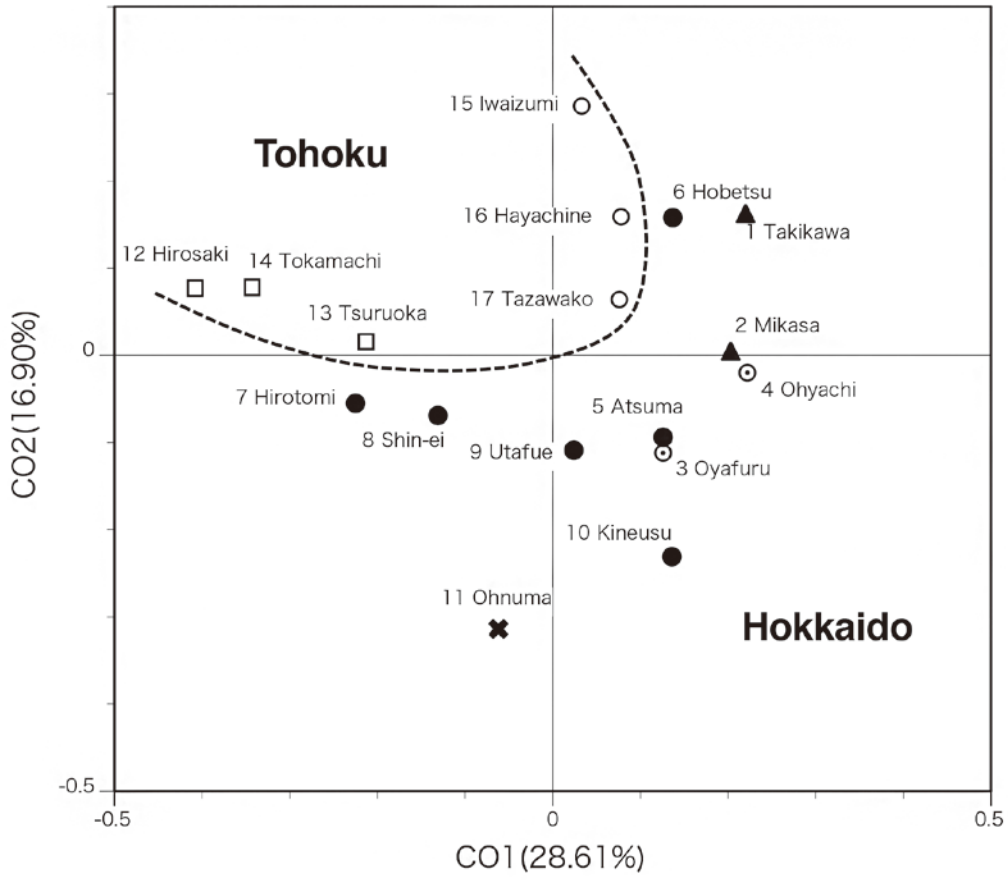

FIG.4. Principal coordinate analysis (PCoA) results for 17 populations of *Q. serrata*. Dotted line divides populations between Tohoku and Hokkaido regions.

0.05) under TPM and SMM (Table 1). On the one hand, significant *P* values were detected under IAM in populations in Hokkaido, except for 1-Takikawa, 6-Hobetsu, and 7-Hirotomi. However, the IAM may include a type I error (Cornuet & Luikart 1996) since its standard deviations were greater than SMM or TPM (not shown).

The IBD among all 17 populations and the 11 populations on Hokkaido were significant (Fig. 2, $R^2 = 0.311$ and 0.652, respectively, $P < 0.001$).

The STRUCTURE results are shown in Fig. 3. The rise in the ln $P(X|K)$ value slowed when $K > 4$ and reached a plateau at $K = 7$. The highest ΔK was observed when $K = 4$, indicating that the most likely number of clusters was 4 (Fig. 3c). Bar plots of $K = 2$–7 are shown in Fig. 3a. The $K = 2$ bar plot distinguished the Japan Sea (yellow in Fig. 3a) and Pacific sides of Tohoku (blue in Fig. 3a), and the Hidaka populations shared both clusters. At $K = 3$, the additional cluster (red in Fig. 3a) was assigned to 11-Ohnuma on the Oshima Peninsula. At $K = 4$, the most likely number of clusters, designated the northernmost cluster (green in Fig. 3a). Two populations in Hidaka, 7-Hirotomi and 8-Shin-ei, shared the same ancestral cluster with populations on the Japan Sea side of Tohoku (Fig. 3a). Furthermore, additional clusters with $K = 5$ were assignable to the Ishikari

Plain, namely, 3-Oyafuru and 4-Ohyachi, and to the Pacific side of Honshu. The Ishikari Plain was distinguished by $K = 6$. Furthermore, a mutual cluster was detected among the northernmost Ishikari Plain, Hidaka and Tohoku populations at $K = 7$. Consideration should be given to the possibility of artificial transportation of seeds of *Q. serrata* to Hokkaido during human migrations, although we do not currently have evidence to discuss the role of humans.

The *F*-values for each cluster, analogous to the traditional F_{ST} values estimated between the common ancestral population and each cluster assumed in the *F*-model, at $K = 4$ were 0.024, 0.030, 0.072, and 0.093 (Fig. 3b). The cluster representing the Pacific side of Tohoku (blue) had the lowest *F*-value, while the cluster with the second lowest *F* (yellow) consisted of populations on the Japan Sea side of Tohoku. The highest *F* (red) was assigned to the Oshima Peninsula. The second highest (green) was assigned to the northernmost region.

Figure 4 shows the PCoA result by pairwise F_{ST}. The first coordinate (CO1, explaining 28.61% of variation) reflects the geographic locations from the northernmost, Ishikari Plain, Japan Sea side of Tohoku and Hidaka, to the Pacific side of Tohoku. The second coordinate (CO2, explaining 16.90% of variation) distinguishes the Hokkaido populations, except for 1-Takikawa and 6-Hobetsu, as negative and the Tohoku populations as positive values.

Discussion

Possibility of interspecific hybridization involving Q. serrata *at the northern limit of its distribution*

Sympatric species of oaks may show introgression among species (Whittemore & Schaal 1991, Lee *et al*. 1996, Ishida *et al*. 2003). Ortego *et al*. (2015) studied populations of six species of sympatric oaks using a demographic approach to determine genetic admixture and hybrid swarms. Even though a pervasive haplotype was shared among sympatric species, the species maintained their morphological and nuclear genetic identity. Similarly, the phenotypes of the species in our study sites appeared to be stable, since we did not find morphologically intermediate individuals.

The results of the STRUCTURE revealed that *Q. serrata* in northern Japan showed genetic differentiation among populations (Fig. 3). In contrast, *Q. crispula* in Hokkaido and Tohoku showed low levels of population differentiation in nuclear SSRs (Ohsawa *et al*. 2011). That is, population differentiation in *Q. serrata* was not the same as in *Q. crispula* in the northern portions of their ranges. Our preliminary analysis using 9 EST-SSR loci detected a substantial distinction between *Q. crispula* and *Q. serrata* on Hokkaido (Matsumoto, unpublished data); STRUCTURE analysis clearly distinguished two species by $K = 2$, in which 8% of the individuals of *Q. serrata* and 1% of the individuals of *Q. crispula* showed evidence of natural hybridization with $q < 0.9$. Okaura *et al*. (2007) shows that *Q. serrata* in Hobetsu corresponds with 6-Hobetsu in our study and *Q. crispula* in Ohnuma was fixed for the cpDNA haplotype II. *Quercus serrata* in Ohnuma, corresponding to 11-Ohnuma, had two haplotypes, I and II, indicating that the composition of the haplotypes of sympatric *Q. crispula* and *Q. serrata* in Ohnuma is not entirely consistent.

The apparent species delimitation in nuclear genomes and the compound structure of organelle genomes among species may be explainable by the higher conspecific nuclear gene flow than organelle gene flow (Currat *et al*. 2008, Petit & Excoffier 2009). Higher conspecific gene flow in our study sites may be supported by phenological observations suggesting infrequent interspecific crossing, since *Q. serrata* flowered two weeks earlier than sympatric *Q. crispula* every year (Namikawa, pers. obs.). We therefore disregard the influence of hybridization with *Q. crispula* and interspecific gene flow between nuclear SSRs.

Another issue is whether the migration and expansion of the distribution of *Q. serrata* was independent from *Q. crispula* at the northern limit of their distribution. From paleobotanical evidence, fossil pollen diagrams showed a rapid expansion of the range of *Quercus* in Hokkaido in

lowland forests after 8,000 years BP (Yasuda & Miyoshi 1998). Morphological similarity of fossil pollen of *Quercus*, however, makes it impossible to distinguish between *Q. crispula* and *Q. serrata* (Hayashi *et al*. 2010). Although the range of distribution of *Q. serrata* and *Q. crispula* currently overlap, their microhabitats differ. *Quercus serrata* dominates steep, south to southwest facing drier slopes where *Q. crispula* dominated forests are absent (Namikawa, unpublished data). We therefore do not assume competition between these two species, nor any effects resulting from their past range expansion.

Genetic diversity of Q. serrata *in the northern range of distribution*

Plant populations on the margins of their range of distribution generally tend to have low genetic diversity (Hampe & Petit 2005, Vernesi *et al*. 2012, Iwasaki *et al*. 2012, Kitamura *et al*. 2015). The southern richness to northern purity of genetic diversity in the northern hemisphere under the influence of the Quaternary ice age is evident (Hewitt 2000). For example, northern populations show less genetic diversity in *Betula maximowicziana* (Tsuda & Ide 2005) and *Fagus sylvatica* (Comps *et al*. 2001) than in their more southern populations. A thorough investigation of *Fagus crenata* showed that genetic diversity declined northward (Kitamura *et al*. 2015). Our findings, however, revealed average heterozygosity of *Q. serrata* on Hokkaido (H_E = 0.694) to be comparable (P = 0.051) to that of the Tohoku populations (H_E = 0.667) (Table 1). Furthermore, the northernmost populations (1-Takikawa and 2-Mikasa) did not show a decline in genetic diversity (P = 0.838). Allowing that our study did not cover the entire range of *Q. serrata*, but was confined to the northern limit of its distribution, our findings are inconsistent with previous studies of major deciduous forest trees in the Japanese Archipelago, whose genetic diversity shows a decline in northern populations (e. g. Tomaru 2013).

That *Q. serrata* in Hokkaido does not show a northward decline in genetic diversity may indicate that the species is not only the result of recent northward expansion, but may have been derived from relicts from earlier distributions, which provided relevant genetic diversity. Recent studies suggest the existence of cryptic refugia (Provan & Bennett 2008) within northern ranges of distribution (Bhagwat & Willis 2008). The existence of relict populations of temperate tree species, such as *B. maximowicziana* (Tsuda & Ide 2010) and *Fraxinus mandshurica* var. *japonica* (Hu *et al*. 2010), in the Japanese Archipelago, has also been discussed. Ohsawa *et al*. (2011) discussed the possibility of cryptic refugia for *Q. crispula*, which is closely related to *Q. serrata*, in Hokkaido. Hence, the existence of northern relict populations of *Q. serrata* may explain the ample amount of genetic diversity among present populations.

The present distribution of *Q. serrata* is characterized by climatic condition on the Pacific Ocean side of Japan. The discontinuous distribution in the Hidaka, Iburi and Oshima regions (Uemura & Takeda 1987) could be due to fragmentation of a formerly continuous distribution by climatic oscillations (Yasuda & Miyoshi 1998). Three extremely small and isolated populations are on Hokkaido; 3-Oyafuru, 6-Hobetsu, and 11-Ohnuma. 3-Oyafuru is a small remnant population with a small number of juveniles of *Q. serrata* on the low Ishikari Plain. 6-Hobetsu has only 30 mature trees and no saplings or seedlings. 11-Ohnuma is also small and isolated from the other populations by more than 100 km (Fig. 1). IBD (Fig. 2) indicates little practical gene flow. These populations have been more prone to recent disturbances caused by human settlement since *Q. serrata* grows at lower elevations and is therefore adjacent to or overlaps with human settlements. The low genetic diversity might be expected due to the extremely small population size and isolation (Young *et al*. 1996, Lowe *et al*. 2005). However, genetic diversity, such as H_E and *AR*, was not significantly different from other populations ($P > 0.05$). We assume that even though these populations had experienced population reduction and isolation, an ample amount of genetic diversity might be secured if the population is kept intact after reduction and isolation (Kitamura *et al*. 2015). In addition, Isagi (2004)

pointed out that long-lived trees do not show reduction in genetic diversity due to recent fragmentation because the effects of the fragmentation on genetic diversity become evident only after a long period of generational turnover. Trees of *Q. serrata* in the Hidaka region are 55 to 100 years old (Ishikawa *et al*. 2015). The maximum age for the species may be comparable with other species of *Quercus*, which ranges from 300 to 400 years (Di Filippo *et al*. 2015). It is probable that the small, isolated populations of *Q. serrata* are the result of fragmentation too recent to be reflected in the loss of genetic diversity.

Genetic structure of Q. serrata *in the northern range of distribution*

Our study revealed several genetic implications for the northern populations of *Q. serrata*. Firstly, we recognized ancestral clusters by STRUCTURE analysis reflecting geographical locations (Fig. 3). The most likely numbers of cluster, $K = 4$, revealed that the lowest (0.024) and the second lowest (0.030) *F*-values represented the Pacific and Japan Sea sides of Tohoku, respectively, indicating that *Q. serrata* in Tohoku is ancestral to the more northerly populations (Fig. 3b). Those clusters were also allocated to Hidaka and the Ishikari Plains, although the Ishikari Plain was assigned to clusters with $K > 5$. The results indicate that *Q. serrata* in Hidaka has conserved the ancestral lineage related to the Tohoku region. Honjo *et al*. (2004) found that *Primula sieboldii* in the Hidaka region shared a common cpDNA haplotype with the Tohoku region and corroborated the Hidaka area as being a refugium for many temperate species during the last glacial period in Hokkaido (Uemura & Takeda 1987).

In contrast, the population on the Oshima Peninsula is highly differentiated for $K > 3$ (Fig. 3). Similarly, localized genetic variation in cpDNA was found on the Oshima Peninsula (Okaura *et al*. 2007). *Euonymus oxyphyllus* also exhibits local genetic variation on the Oshima Peninsula (Iwasaki *et al*. 2012). It is evident that the differentiation of 11-Ohnuma population can be explained by IBD (Fig. 2). However, STRUCTURE analysis showed that the cluster representing the Oshima Peninsula, red in Fig. 3a, was also present in the Hidaka populations. More significantly, the PCoA results support differentiation of the Hokkaido and Tohoku populations, and the population in Oshima Peninsula being classified among Hokkaido (Fig 4). Our findings indicate that the 11-Ohnuma population is closer to other populations on Hokkaido than to those in the Tohoku region.

We thank Y. Tsumura, M. Kobayashi, and M. Ooue for supplying material from the Tohoku region. We also thank the members of the Iwate Shokubutsu no Kai, Y. Honma, T. Nagamitsu, T. Matsui, T. Yagihashi, M. Sasaki, K. Ogami, K. Uemura, H. Suzuki, A. Takazawa, and Y. Sakamoto for their support and discussion. This work was supported by the President Discretionary Grant for Scientific Research, the Hokkaido University of Education to KN, and partly supported by a grant from the Forestry Agency to AM and LSJ-M.

References

Bhagwat, S. A. & K. J. Willis. 2008. Species persistence in northerly glacial refugia of Europe: a matter of chance or biogeographical traits? J. Biogeogr. 35: 464–482.

Comes, H. P. & J. W. Kadereit. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. Trends Pl. Sci. 3: 433–438.

Comps, B., D. Gömöry, J. Letouzey, B. Thiébaut & R. J. Petit. 2001. Diverging trends between heterozygosity and allelic richness during postglacial colonization in the European beech. Genetics 157: 389–397.

Cornuet, J. M. & G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001–2014.

Currat, M., M. Ruedi, R. J. Petit & L. Excoffier. 2008. The hidden side of invasions: massive introgression by local genes. Evolution 62: 1908–1920.

Di Filippo, A., N. Pederson, M. Baliva, M. Brunetti, A. Dinella, K. Kitamura, H. D. Knap, B. Schirone & G. Piovesan. 2015. The longevity of broadleaf deciduous trees in northern hemisphere temperate forests: insights from tree-ring series. Frontiers in Ecol. Evol. 3: 46. doi: 10.3389/fevo.2015.00046.

Earl, D. A. & B. M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genet. Res. 4: 359–361.

Excoffier, L., G. Laval & S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for popula-

tion genetics data analysis. Evol. Bioinf. Online 1: 47–50.

Goudet, J. 1995. F-stat version 1.2: a computer program to calculate F-statistics. J. Hered. 86: 485-486. Available from http://www2.unil.ch/popgen/softwares/fstat.htm [accessed 23 November 2010]

Hampe, A. & R. J. Petit. 2005. Conserving biodiversity under climate change: the rear edge matters. Ecol. Letters 8: 461–467.

Harada, K & S. Ikeda. 2011. Molecular population genetic study of *Quercus mongolica* var. *crispula* (Fagaceae) using methionine synthase gene (*MetE*) in Japanese Archipelago. Forest Tree Breed. 241: 1–11 (in Japanese with English summary).

Hayashi, R., J. Inoue, M. Makino & H. Takahara. 2010. Vegetation history during the last 17,000 years around Sonenuma Swamp in the eastern shore area of Lake Biwa, western Japan: With special reference to changes in species composition of *Quercus* subgenus *Lepidobalanus* trees based on SEM pollen morphology. Quatern. Int. 254: 99–106.

Hedrick, P. W. 2005. A standardized genetic differentiation measure. Evolution 59: 1633–1638.

Heuertz, M., J. F. Hausman, O. J. Hardy, G. G. Vendramin, N. Franscaria-Lacoste & X. Vekemans. 2004. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and southeastern European populations of the common ash (*Fraxinus excelsior* L.). Evolution 58: 976–988.

Hewitt, G. M. 2000. The genetic legacy of the Quaternary ice ages. Nature 405: 907–913.

Honjo, M., S. Ueno, Y. Tsumura, I. Washitani & R. Ohsawa. 2004. Phylogeographic study based on intraspecific sequence variation of chloroplast DNA for the conservation of genetic diversity in the Japanese endangered species *Primula sieboldii*. Biol. Conservation 120: 211–220.

Hu, L. -J., K. Uchiyama, Y. Saito & Y. Ide. 2010. Contrasting patterns of nuclear microsatellite genetic structure of *Fraxinus mandshurica* var. *japonica* between northern and southern populations in Japan. J. Biogeogr. 37: 1131–1143.

Isagi, Y. 2004. Characteristics of regeneration processes of tree species occurring at low density revealed by microsatellite markers. J. Jap. Forest Soc. 86: 169–176. (in Japanese with English summary)

Ishida, T. A., K. Hattori, H. Sato & M. T. Kimura. 2003. Differentiation and hybridization between *Quercus crispula* and *Q. dentata* (Fagaceae): insights from morphological traits, amplified fragment length polymorphism markers, and leaf miner composition. Amer. J. Bot. 90: 769–776.

Ishikawa, Y., K. Yabe & H. Yamagishi. 2015. Establishment and growth of the *Alnus japonica* forest at the northwestern coast of Lake Utonai in the southern area of Ishikari Depression, northern Japan. Vegetation Sci. 32 : 81– 94. (in Japanese with English summary)

Iwasaki, T., K. Aoki, A. Seo & N. Murakami. 2012. Comparative phylogeography of four component species of deciduous broad-leaved forests in Japan based on chloroplast DNA variation. J. Pl. Res. 125: 207–221.

Jakobsson, M. & N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23: 1801–1806.

Kanno, M. 2004. Phylogeography and population genetic study of sect. Prinus of genus *Quercus* (Fagaceae) in Japan. PhD Thesis, Graduate School of Life Science, University of Tohoku, Miyagi.

Kanno, M., J. Yokoyama, Y. Suyama, M. Ohyama, T. Itoh & M. Suzuki. 2004. Geographical distribution of two haplotypes of chloroplast DNA in four oak species (*Quercus*) in Japan. J. Pl. Res. 117: 311–317.

Kitamura, K., T. Matsui, M. Kobayashi, H. Saitou, K. Namikawa & Y. Tsuda. 2015. Decline in gene diversity and strong genetic drift in the northward-expanding marginal populations of *Fagus crenata*. Tree Genet Genomes 11: 36. doi: 10.1007/s11295-015-0857-y

Lascoux, M., A. E. Palme, R. Cheddadi & R. G. Latta. 2004. Impact of Ice Ages on the genetic structure of trees and shrubs. Philos. Trans. Ser. B 359: 197–207.

Lawton, J. H. 1993. Range, population abundance and conservation. Trends Ecol. Evol. 8: 409–413.

Lee, J. H., H. Hashizume & F. Yamamoto. 1996. Variations in the flowering time, pollen morphology and fertility of *Quercus dentata* Thunb., *Q. serrata* Thunb., *Q. mongolica* Fischer var. *grosseserrata* Rehder et Wilson and their intermediate types. J. Jap. Forest Soc. 78: 452–456 (in Japanese with English summary).

Lowe, A. J., D. Boshier, M. Ward, C. F. E. Bacles & C. Navarro. 2005. Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity 95: 255–273.

Lumaret, R., M. Typhon-Dionnet, H. A. Michaud, E. Ipotesi, C. Born & C. Mir. 2005. Phylogeographical variation of chloroplast DNA in cork oak (*Quercus suber*). Ann. Bot. (Oxford) 96: 853–886.

Magni, C. R., A. Ducousso, H. Caron, R. J. Petit & A. Kremer. 2005. Chloroplast DNA variation of *Quercus rubra* L. in North America and comparison with other Fagaceae. Molec. Ecol. 14: 513–524.

Magri, D., G. G. Vendramin, B. Comps, I. Dupanloup, T. Geburek, D. Gömöry, M. Latalowa, T. Litt, L. Paule, J. M. Roure, I. Tantau, W. O. van der Knaap, R. J. Petit & J. -L. de Beaulieu. 2006. A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences.

New Phytol. 171: 199–221.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209–220.

McLachlan, J. S., J. S. Clark & P. S. Manos. 2005. Molecular indicators of tree migration capacity under rapid climate change. Ecology 86: 2088–2098.

Meirmans, P. G. & P. H. Van Tienderen. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms, Molec. Ecol. Notes. 4: 792–794.

Mishima, K., A. Watanabe, K. Isoda, M. Ubukata & K. Tanaka. 2006. Isolation and characterization of microsatellite loci from *Quercus mongolica* var. *crispula*. Molec. Ecol. Notes 6: 695–697.

Ohsawa, T., Y. Tsuda, Y. Saito & Y. Ide. 2011. The genetic structure of *Quercus crispula* in northeastern Japan as revealed by nuclear simple sequence repeat loci. J. Pl. Res. 124: 645–654.

Okaura, T., N. D. Quang, M. Ubukata & K. Harada. 2007. Phylogeographic structure and late Quaternary population history of the Japanese oak *Quercus mongolica* var. *crispula* and related species revealed by chloroplast DNA variation. Genes Genet. Systems 82: 465–477.

Ortego, J. R., V. Noguerales, P. F. Gugger & V. L. Sork. 2015. Evolutionary and demographic history of the Californian scrub white oak species complex: an integrative approach. Molec. Eco. 24: 6188–6208.

Peakall, R. & P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molec. Ecol. Notes 6: 288–295. Available from http://biology.anu.edu.au/GenAlEx/Welcome.html [accessed 30 August 2012]

Petit, R. J., U. M. Csaikl, S. Bordács, K. Burg, E. Coart, J. Cottrell, B. C. van Dam, J. D. Deans, S. Dumolin-Lapègue, S. Fineschi, R. Finkelday, A. Gillies, I. Glaz, P. G. Goicoechea, J. S. Jensen, A. König, A. J. Lowe, S. F. Madsen, G. Mátyás, R. C. Munro, M. Olalde, M. -H. Pemonge, F. Popescu, D. Slade, H. Tabbener, D. Taurchini, S. M. G. de Vries, B. Ziegenhagen & A. Kremer. 2002. Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2600 populations. Forest Ecol. Managem. 156: 5–26.

Petit, R. J. & L. Excoffier. 2009. Gene flow and species delimitation. Trends Ecol. Evol. 24: 386–393.

Piry, S., G. Luikart & J. M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J. Heredity 90: 502–503. Available from http://www.ensam.inra.fr/URLB/bottleneck/bottleneck.html [accessed 23 November 2010]

Pritchard, J. K., M. Stephens & P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959. Available from http://pritch.bsd.uchicago.edu/structure.html [accessed 5 December 2010]

Provan, J. & K. D. Bennet. 2008. Phylogeographic insight into cryptic glacial refugia. Trends Ecol. Evol. 23: 564–571.

Quang, N. D., S. Ikeda & K. Harada. 2008. Nucleotide variation in *Quercus crispula* Blume. Heredity 101: 166–174.

Steinkellner, H., S. Fluch, E. Turetschek, C. Lexer, R. Streiff, A. Kremer, K. Burg & J. Glössl. 1997. Identification and characterization of (GA/CT)n - microsatellite loci from *Quercus petraea*. Pl. Molec. Biol. 33: 1093–1096.

Stewart, J. R. & A. M. Lister. 2001. Cryptic northern refugia and the origins of the modern biota. Trends Ecol. Evol. 16: 608–613.

Taberlet, P., L. Fumagalli, A. -G. Wust-Saucy & J. -F. Cosson. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molec. Ecol. 7: 453–464.

Tohyama, M. & Y. Mochida. 1978. Deciduous broad-leaf forests in eastern Iburi, Hokkaido. Papers on plant ecology to the memory of Dr. Kuniji Yoshioka. Sendai. p.134–149 (in Japanese, title were translated by the authors).

Tomaru, N. 2013. Genetic diversity and population genetic structure in widespread deciduous broad-leaved trees in Japan. Chikyu Kankyo 18: 119–126. (in Japanese)

Tsuda, Y. & Y. Ide. 2005. Wide-range analysis of genetic structure of *Betula maximowicziana*, a long-lived pioneer tree species and noble hardwood in the cool temperate zone of Japan. Molec. Ecol. 14: 3929–3941.

Tsuda, Y. & Y. Ide 2010. Chloroplast DNA phylogeography of *Betula maximowicziana*, a long-lived pioneer tree species and noble hardwood in Japan. J. Pl. Res. 123: 343–353.

Ubukata, M. 2003. Ecological genetic study on the genetic resources and natural forest management of Mizunara (*Quercus mongolica* var. *grosseserrata*) in Hokkaido. Bull. Forest Tree Breed. Center 19: 25–120. (in Japanese)

Uemura, S. & Y. Takeda. 1987. Phytogeographical study on the distribution of the main temperate plants composing the natural forests in Hokkaido, Japan. *In*: The Kobe Geobotanical Society (eds.) Papers on plant ecology and taxonomy to the memory of Dr. Satoshi Nakanishi. p.255–269 (in Japanese with English summary).

Vernesi, C., D. Rocchini, E. Pecchioli, M. Neteler, G. G. Vendramin & D. Paffetti. 2012. A landscape genetics approach reveals ecological-based differentiation in populations of holm oak (*Quercus ilex* L.) at the northern limit of its range. Biol. J. Lin. Soc. 107: 458–467.

Whittemore, A. T. & B. A. Schaal. 1991. Interspecific

gene flow in sympatric oaks. Proc. Natl. Acad. Sci. U.S.A. 88: 2540–2544.

Yasuda, Y. & N. Miyoshi. 1998. Vegetation History of Japanese Archipelago. Asakura Shoten, Tokyo. (in Japanese)

Young, A., T. Boyle & T. Brown. 1996. The population genetic consequences of habitat fragmentation for plants. Trends Ecol. Evol. 11: 413–418.

Received November 19, 2015; accepted September 6, 2016